Sewage Sludge and Pisolithus tinctorius *Ectomycorrhizae: Their Effect on Growth of Pine Seedlings*

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Abstract. Four levels of dried sludge (0, 69, 138, and 275 metric tons/ha) and infestation of soil with the fungal symbiont *Pisolithus tinctorius* or control inoculum were combined factorially in heavy clay soil from a severely eroded site to determine their effects on growth of *Pinus taeda* and *P. echinata* seedlings. Addition of sludge to soil caused highly significant increases in growth of both pine species. Seedlings of both pine species with *Pisolithus* ectomycorrhizae were significantly taller than those with ectomycorrhizae formed by naturally occurring fungi. Loblolly pine seedlings with *Pisolithus* ectomycorrhizae also had significantly greater stem diameters and foliar and root fresh weights than control seedlings. Foliar zinc increased and foliar aluminum decreased with increased sludge levels. Loblolly seedlings with *Pisolithus* ectomycorrhizae also had significantly less foliar zinc than control seedlings. There were no correlations between sludge levels or *Pisolithus* ectomycorrhizae and amounts of 13 other elements in the foliage. The effects of *Pisolithus* ectomycorrhizae were greatest at the intermediate sludge level (138 tons/ha) on both loblolly and shortleaf pine seedlings. Based on these results, the interaction of dried sewage sludge with *Pisolithus* ectomycorrhizae appears to have valuable potential in the improvement of pine growth on eroded forest sites. ***Forest Sci. 22:351–358.***

Additional key words. Nutritional disorder, site reclamation, *Pinus taeda*, *Pinus echinata*.

THE DEPENDENCE OF PINES on ectomycorrhizal fungi is well known (Marks and Kozlowski 1973). In addition, Marx and others (1970) and Marx and Bryan (1971) demonstrated that certain of these fungi are more beneficial to growth of southern pines than others. *Pisolithus tinctorius* (Pers) Coker and Couch, in particular, is able to synthesize ectomycorrhizae and promote rapid growth of pines under adverse environmental conditions, such as high soil temperatures, minimal soil fertility, and soil reactions as acid as pH 3.5, conditions often found on strip-mined coal spoils (Schramm 1966). These and other observations indicate the feasibility of inoculating pine seedlings with specific mycorrhizal fungi which would promote survival and growth on sites having a variety of adverse soil conditions.

In the Piedmont region of South Carolina, Georgia, and Alabama there are about two million hectares of severely eroded forest sites caused by previous poor agricultural practices (Campbell and others 1953). These sites, with soils in the Cecil-Madison series, are characterized by a thin to absent topsoil underlain by a heavy, plastic, and poorly drained clay subsoil. The poor soils on these sites severely limit tree growth and are those on which littleleaf disease of shortleaf (*Pinus echinata* Mill.) and loblolly (*P. taeda* L.) pines occur (Zak 1961).

Sewage sludge has been used as a soil

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amendment to promote revegetation of low-fertility, strip-mined mineral spoils. Lejcher and Kunkle (1973) found that 304 dry metric tons/hectare of sludge were sufficient to support a 90 percent cover of K-31 tall fescue (*Festuca arundanacea*) and weeping love grass (*Eragrostis curvula*) for at least two growing seasons on a coal spoil. Sopper and others (1970) obtained 80 percent survival of black locust (*Robinia pseudoaccacia* L.) on coal spoil material after application of 1.1 to 2.2 dry metric tons/ha of sewage effluent and 6.4 to 12.8 dry metric tons/ha of sludge. Most locust trees planted on the nonamended coal spoil died.

The value of specific ectomycorrhizal fungi to trees growing in soil amended with sewage sludge has not been investigated. Merrill (1969) found no differences in ectomycorrhizal development of *Pinus resinosa* in nonirrigated stands and those irrigated with sewage effluent. He did not, however, determine possible differences in species of fungi forming the ectomycorrhizae in the two groups of trees.

Recently, techniques were developed for "tailoring" roots of pines growing in nursery beds with mycorrhizae formed by *Pisolithus tinctorius*, an ectomycorrhizal symbiont ecologically adapted to adverse sites (Marx and Bryan 1975). By combining the techniques of "tailoring" seedlings and amending soil with sludge, we may significantly increase both survival and growth of pines on sites having eroded or low-fertility soils conducive to littleleaf disease. This paper reports the effects of such treatments on the growth of loblolly and shortleaf pine seedlings in clay soil taken from a typical littleleaf disease site.

Materials and Methods

A completely randomized factorial experiment involving 4 levels of sludge × 2 ectomycorrhizal conditions × 3 replications was installed in 24 microplots. One-half of each microplot was planted with loblolly pine and one-half with shortleaf pine. The microplots were constructed of 5 × 30 cm redwood planks as above-ground rectangular frames with dimensions of 100 × 150 × 30 cm and a volume of 450 liters. The microplots were placed approximately 1 m apart on a recently tilled and leveled site.

TABLE 1. Plant nutrients and organic matter in dried sludge from sewage treatment plants at Athens, Georgia.[a]

Constituent element	Acid extractable[b]	Total[c]
	p.p.m.	*p.p.m.*
N	—	21,500
NO_3-N	3,613	—
P	161	9,034
K	41	4,034
Ca	580	13,750
Mg	147	284
Zn	205	1,105
B	1.14	17.5
Mn	59	133
Fe	101	7,608
Na	205	344
Cu	18	414
Al	134	5,945
Organic matter (percent)		(48.9)

[a] Analyses performed by the Soil Testing Laboratory, Cooperative Extension Service, University of Georgia, Athens 30602. Each value is the average of six analyses.

[b] Double acid extraction method (0.05 N HCl in 0.025 N H_2SO_4).

[c] Total nitrogen analyzed by Kjeldahl method; organic matter determined by ashing; and metallic elements determined by atomic absorption spectroscopy.

Dried sewage sludge was obtained from two primary sewage treatment plants in Athens, Georgia, which utilize the anaerobic digestion process. The acid-extractable (double acid extraction, Anonymous 1974) and total quantities of 12 nutrient elements and percent organic matter were measured in three samples from each plant (Table 1). Analyses did not reveal significant differences between sludges from the two sources, so sludge from the two sources was combined. The total values are comparable to those of King and Morris (1972) for liquid sludge from the same sewage treatment plants. Quantities of elements extracted by double acid extraction are considered to be estimates of the amounts most readily available to plants.

Heavy red clay soil of the Cecil-Madison series, low in organic matter and nutrients, was removed from a typical littleleaf disease site in the Georgia Piedmont and screened to 0.5 cm particle size. One of four levels of dried sewage sludge was added to the soil in each of six microplots, establishing sludge rates of 0, 69, 138, and 275 metric tons per ha, respectively. All microplots were filled to within 5.0 cm of the top. The soil and sludge were thoroughly mixed in the frames with a rototiller.

In April 1973, vegetative mycelial inoculum of *P. tinctorius* (isolate 55) was broadcast on the surface of three microplots per sludge level. Autoclaved inoculum was similarly added to the remaining three microplots of each sludge treatment for controls. Inoculum preparation and amount used to infest soil have been described (Marx and Bryan 1975). Inoculum was thoroughly mixed to a depth of 15 to 18 cm by rototilling, and the soil surface was leveled.

Loblolly and shortleaf pine seeds were soaked for 48 hours in one percent H_2O_2 at 5° C to break dormancy and then surface sterilized for 15 min at room temperature in 30 percent H_2O_2. Ten seeds were planted at each of 126 intersects on a 10 × 10 cm grid on the soil surface of each microplot. Half of each microplot was planted with loblolly pine seed and the other half with shortleaf pine seed in a split-plot design. After planting, the microplots were mulched to a depth of approximately 1 cm with fumigated pine straw and covered with plastic screen to protect the germinating seed from birds and mammals. The screen was removed one month later, and seedlings were thinned to one per position. In some instances, seedlings were transplanted to vacant positions left by nongerminated seed. Sixty-three loblolly pines and 63 shortleaf pines were established in each microplot. Seedlings received overhead irrigation as needed during the growing season.

The study was terminated 8 months after seed germination. Five 100 ml subsamples of soil were removed from each pine species subplot in each microplot, and combined parts of each sample were analyzed chemically for major and minor elements and assayed for nematodes, saprophytic fungi, and pythiaceous fungal pathogens. Bacteria were isolated on peptone dextrose-rose bengal agar at $1{:}10^5$ and $1{:}10^6$ dilutions and saprophytic fungi on soil extract agar at dilutions of $1{:}10^4$ and $1{:}10^5$ (Johnson and Curl 1972).[1] Populations of pythiaceous fungi were isolated on modified Kerr's (Hendrix and Kuhlman 1965) and Flowers and Hendrix' (1969) agar media using the methods developed by Marx (1973). Plant-parasitic nematodes were extracted from soil by Jenkins' (1964) centrifugal-flotation technique.[2]

Seedlings were removed from the soil by first removing the microplot frame and lifting the roots gently with a shovel. All seedlings, including border trees, were evaluated for fusiform rust (*Cronartium fusiforme* Hedgc. and Hunt). After border row trees were removed, 10 rust-free seedlings of each pine species per microplot were randomly selected for measurements of height, stem diameter at root collar, and shoot and root fresh weights. Percentages of roots ectomycorrhizal with *P. tinctorius* and other symbiotic fungi were visually estimated. After the seedlings were weighed, secondary needles were removed and combined into one sample per subplot. Foliar analyses were made for all major and minor nutrients. *Pisolithus* ectomycorrhizae were surface sterilized and placed on agar slants for reisolation of *Pisolithus* (Marx and Bryan 1975). Data were subjected to analyses of variance, and means were separated by least significant differences (LSD) at $P = 0.05$. The test for quadratic effects was made by means of a single degree of freedom contrast with an equal spacing for the levels of sludge utilizing the T statistics at $P = 0.05$.

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[1] Fungi were identified by William J. Otrosina, Graduate Research Assistant, School of Forest Resources, University of Georgia, Athens 30602.

[2] Nematode extraction and identification was by Dr. John Ruehle, USDA Forest Service, Forestry Sciences Laboratory, Athens, Georgia 30602.

TABLE 2. Nutrient and organic matter content of mixtures of clay soil and sewage sludge after growing pine seedlings for 8 months.[a] *Values are means of six samples.*

Sludge rate (metric ton/ha)	pH	NO_3-N	Total N	P	K	Ca	Mg	Zn	Organic matter
			– – – – – – – – – – *p.p.m.* – – – – – – – – – –						*Percent*
0	5.2	1	550	0	37	105	18	1.5	0.6
69	4.7	12	800	11	30	157	15	7.5	1.3
138	4.7	14	1100	16	27	190	14	12.8	1.8
275	4.2	30	2250	54	23	246	7	22.8	3.8

[a] Analyses performed by the Soil Testing Laboratory, Cooperative Extension Service, University of Georgia, Athens 30602.

Results

Chemical analyses of soil from all microplots (Table 2) showed that major elements and organic matter were low in the nonamended clay soil at the end of the study. With the exception of potassium and magnesium, all elements were higher in the sludge treatments than in nonamended soil. Neither *Pisolithus* nor pine species affected the chemical status of the soils.

All growth parameters of seedlings were significantly greater in the sludge treatments than in the nonamended soil (Tables 3,4). This stimulation of seedling growth, however, was quadratic in that the 138 ton/ha level of sludge was more stimulating to growth than either the 69 or 275 ton/ha levels.

Loblolly and shortleaf pine seedlings with *Pisolithus* ectomycorrhizae were larger than control seedlings at the 138 ton/ha sludge level. Infestation of soil with *Pisolithus* did not result in a greater total number of ectomycorrhizae in any treatment where sludge was applied, but it did significantly increase the total ectomycorrhizal development on loblolly in the nonamended soil. *Pisolithus* was reisolated from 45, 32, 23 and 12 percent of the surface-sterilized ectomycorrhizae of both pine species in the 0, 69, 138, and 275 ton/ha sludge levels, respectively. The rates of successful reiso-

TABLE 3. Growth and ectomycorrhizal development of loblolly pine seedlings after 8 months in a heavy clay soil amended with sewage sludge and inoculum of Pisolithus tinctorius *(Pt). Each number is the mean of 10 seedlings from each of 3 microplots.*

Soil infestation treatment	Sludge rate (metric ton/ha)	Height	Stem diam	Fresh wt			Shoot-root ratio	Proportion of roots ectomycorrhizal with—			Seedlings with fusiform rust[a]
				Shoot	Root	Total		Pt	Other	Total	
		cm	*mm*	*gm*	*gm*	*gm*		*Percent*	*Percent*	*Percent*	*Percent*
Pisolithus tinctorius	0	13.9	3.1	3.2	2.5	5.7	0.83	24	38	62	14
	69	22.7	5.7	14.0	7.5	21.5	.54	43	31	74	15
	138	27.2	7.4	22.2	11.7	33.9	.53	46	18	64	12
	275	23.7	6.7	16.5	9.7	26.2	.59	38	20	58	16
Control	0	12.7	2.7	2.8	2.0	4.8	.79	0	35	35	14
	69	21.8	5.7	13.6	6.9	20.5	.52	0	71	71	13
	138	23.7	6.3	16.8	8.9	25.7	.53	0	59	59	10
	275	21.0	5.9	13.3	8.9	22.2	.66	0	61	61	19
LSD	(0.05)	3.3	0.9	3.8	1.9	5.3	0.18			19	6

[a] Based on all surviving seedlings in each microplot.

TABLE 4. Growth and ectomycorrhizal development of shortleaf pine seedlings after 8 months in a heavy clay soil amended with sewage sludge and inoculum of Pisolithus tinctorius *(Pt). Each number is mean of 10 seedlings from each of 3 microplots.*

Soil infestation treatment	Sludge rate (metric ton/ha)	Height	Stem diam	Fresh wt			Shoot-root ratio	Proportion of roots ectomycorrhizal with—		
				Shoot	Root	Total		Pt	Other	Total
		cm	*mm*	*gm*	*gm*	*gm*		*Per-cent*	*Per-cent*	*Per-cent*
Pisolithus tinctorius	0	8.3	2.1	1.5	1.3	2.7	0.92	41	22	63
	69	13.4	4.2	6.3	4.6	10.9	.74	50	18	68
	138	14.0	4.8	10.0	7.1	17.1	.72	57	17	74
	275	12.6	4.4	8.4	5.3	13.7	.63	56	8	64
Control	0	7.5	2.0	1.5	1.2	2.7	.84	0	49	49
	69	11.7	3.9	6.0	3.8	9.7	.63	0	64	64
	138	10.6	4.1	5.8	4.3	10.1	.74	0	63	63
	275	10.2	4.0	6.3	4.9	11.2	.82	0	60	60
LSD	(0.05)	2.7	1.1	3.5	2.2	5.6	0.20			19

lation of *P. tinctorius* in the sludge treatments were inversely related to frequencies of bacterial contamination of the surface-sterilized ectomycorrhizae.

Chemical analyses of needles of both pine species showed that only Zn and Al were significantly affected by either sludge or *Pisolithus* treatment (Table 5). Other elements (N, P, K, Ca, Mg, Mn, Fe, B, Cu, Mo, Sr, Ba and Na) were not affected. Zinc concentrations were positively correlated and Al concentrations were negatively correlated with increasing sludge levels. At all sludge levels there was significantly less Zn in the needles of loblolly pines with *Pisolithus* ectomycorrhizae than in seedlings ectomycorrhizal by naturally occurring symbionts.

Populations of saprophytic fungi and pythiaceous fungal pathogens were not significantly influenced by sludge treatments. *Pythium irregulare* Buis. was the only root pathogen isolated. It was probably indigenous in the clay soil, since we

TABLE 5. Concentrations of aluminum and zinc in foliage of loblolly and shortleaf pine seedlings grown in clay soil amended with sewage sludge and inoculum of Pisolithus tinctorius.

Soil infestation treatment	Sludge rate (metric ton/ha)	Loblolly		Shortleaf	
		Zinc	Aluminum	Zinc	Aluminum
		p.p.m. (dry weight basis)			
Pisolithus tinctorius	0	65	1438	66	1990
	69	99	1409	163	1855
	138	143	895	160	456
	275	268	749	262	735
Control	0	70	1601	72	1640
	69	152	1212	150	1099
	138	215	880	239	1475
	275	297	1080	300	1120
LSD	(0.05)	48	541	89	845

failed in several attempts to isolate it from nondiluted sludge. The saprophytic fungi most frequently obtained were *Trichoderma* spp. and *Penicillium* spp. Other genera were *Chrysosporium*, *Cephalosporium*, *Pestalotia*, *Epicoccum*, *Trichocladium*, *Mucor*, *Paecilomyces*, *Gliocladium*, *Nigrospora*, *Fusarium*, *Monosporium*, and *Coniothyrium*. The occurrence of these fungi or their population densities were not related to sludge levels or mycorrhizal treatments.

Neither sludge nor *Pisolithus* treatments significantly affected populations of saprophytic or plant-parasitic nematodes. Of the latter, only low numbers of ring (*Criconemoides* spp.), dagger (*Xiphinema americanum* Cobb.), and spiral (*Helicotylenchus* spp.) nematodes were recovered. These species are normal constituents of the microfauna of littleleaf sites (Ruehle 1962) and were apparently introduced with the clay soil. Sludge alone was not assayed for nematodes.

Fusiform rust galls were found on loblolly seedlings in all treatments, with the fewest occurring on seedlings without *Pisolithus* ectomycorrhizae in the 138 ton/ha sludge level.

Discussion

The properties of sewage sludge may vary considerably with the area and treatment process, according to Peterson and others (1973). They found that different sources of sludge varied on a percent dry weight basis from 3.5 to 6.3 for total N, 0.75 to 3.9 for P, and 0.22 to 0.68 for K. According to Dean and Smith (1973), concentrations of trace elements also vary widely in sludge. Therefore, rates of application and specific uses for sludge would vary from area to area. The application of dried sewage sludge to a disturbed site to support tree growth should be as heavy as environmental considerations and the economics of transportation and application will permit. Unlike agronomic conditions, only one application of sludge per "tree crop" rotation cycle is likely to be made. This single application ideally should be heavy enough to provide a reservoir of organic matter which with decomposition would slowly release nitrogen and other essential elements for several years. Additional benefits associated with sludge application would be an increase in soil tilth and moisture holding capacity.

Agricultural benefits of sludge from sewage plants in Athens, Georgia, were studied by King and Morris (1972), who reported nutrient values comparable to those found in our analyses. The potential effects of sludge on soil fertility are indicated by the analyses in Table 1. In the soil mixtures, all levels of sludge increased NO_3-N, P, Ca, and organic matter (Table 2). Concentration of two elements, K and Mg (minor constituents of sludge), were somewhat lower in sludge-soil mixtures than in either the nonamended clay soil or the sludge as determined by acid extraction analyses. This was probably due to increased absolute absorption of these elements, which was proportional to seedling growth.

The overall effect of sludge on seedling growth was striking. There were four- to six-fold increases in total weight of seedlings in the 138 ton/ha sludge level when compared to the nonamended controls (Tables 3 and 4). There were also significant increases in size and weight of seedlings with *Pisolithus* ectomycorrhizae, especially in loblolly pine. Stimulation of growth by *Pisolithus* was less in shortleaf than in loblolly pine. There were no significant interactions between the sludge and mycorrhizal treatments. It is interesting, however, that significant growth effects resulting from *Pisolithus* ectomycorrhizae occurred only at the 138 ton/ha sludge level.

Several other mycorrhizal fungi were observed on seedling roots in addition to *P. tinctorius*. *Thelephora terrestris* was identified from basidiocarps and *Cenococcum graniforme* (Sow.) Ferd. and Winge from its unique jet-black ectomycorrhizae. Other mycorrhizal fungi were rare, and their effects were not regarded as biologically significant.

The ratio of root weight to foliar-stem weight decreased as sludge levels increased with loblolly pine. This growth effect is similar to that expected from application of N and P from commercial fertilizers.

Increased levels of sludge in the soil resulted in an increase in foliar zinc and a decrease in foliar aluminum. No other foliar elements differed noticeably between treatments. The most interesting aspect of the foliar analyses was the significantly lower amount of zinc in foliage of loblolly pine seedlings with *Pisolithus* ectomycorrhizae than in seedlings with naturally occurring ectomycorrhizae. Additional research will be needed to determine the significance of this phenomenon.

An extensive investigation on effects of sludge on soil fungus and bacterial populations was beyond the scope of this study. This work, however, indicated that populations of saprophytic fungi, as well as the root pathogen *Pythium irregulare*, survived in soil following sludge applications. Cooke (1969, 1971), in more extensive studies, found an increase of fungal populations associated with applications of sludge.

We have no explanation for the increased fusiform rust infection on seedlings grown in the 138 ton/ha sludge level. However, in view of the stimulation of growth of both pine species by *Pisolithus* ectomycorrhizae at this sludge level, additional research is warranted.

This study demonstrated that initial growth of loblolly and shortleaf pine seedlings can be greatly stimulated by a single heavy application of dried sewage sludge. It is our tentative conclusion that sludge would be a useful amendment for the rehabilitation of the 2,000,000 severely eroded hectares of forest sites in the southeastern Piedmont region and, perhaps, also for many of the thousands of hectares of land severely disturbed by strip mining. The beneficial effect of the ectomycorrhizal fungus *Pisolithus tinctorius* on growth of pines has already been shown (Marx and Bryan 1971, 1975), but results of this study add additional evidence for the value of this fungal symbiont to growth of pines in poor soils that have been properly amended.

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